Dual Inhibition of PI3K and mTORC1/C2 by PKI-587 (PF-05212384) as a Promising Therapeutic Option for Bronchopulmonary Neuroendocrine Tumor Disease

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Background
Bronchopulmonary neuroendocrine tumors (BP-NETs) differ in their clinical behavior, pathology and prognosis from the more common lung cancer populations. A promising therapy is the targeting of the PI3K/AKT/mTOR protein pathway, which plays a key role in cell proliferation, growth and survival. One substance of this group is the mTOR inhibitor Everolimus (RAD001), which has already shown antiproliferative effects in vitro on BP-NETs cells (1). Clinical Phase III studies like RADJANT 4 (2) have shown beneficial effects in vivo and the approval of this substance for well differentiated BP-NETs is awaited. Nevertheless there are some drawbacks, since objective responses are seldom. The dual inhibitory effect on the PI3K/mTOR pathway has already been tested on several gastroenteropancreatic neuroendocrine tumor (GEP-NEt) cell lines. Here, treatment with PKI-587 was superior to RAD001 treatment, concluding that the dual inhibition of PI3K and mTOR elicits a stronger antiproliferative effect (3).

Methods

Determination of Cell Viability with MTS Cell Proliferation Assay (Promega)

In both cell lines, PKI-587 shows a stronger inhibitory effect than RAD001. However, the mere presence of RAD001 leads to a constant lower cell viability (vs. control), which rapidly decreases with increasing concentration. In contrast to that, small amounts of PKI-587 lead to a slight increase of cell viability (vs. control) which then decreases at different concentrations.

Detection of Apoptosis with Caspase-Glo® 3/7 Assay (Promega)

Caspase 3 and 7 play key effector roles in apoptosis of mammalian cells. To investigate the effect of RAD001 and PKI-587 on apoptosis induction in NCI-H727 and NCI-H69, both cell lines were treated with IC50 and IC90 concentrations of each inhibitor and incubated for 24h. Adding Caspase Glo® 3/7 Reagent to the cells led to generation of a luminescent signal proportional to caspase 3/7 activity, which was afterwards detected by a luminometer.

Conclusion

In both BP-NEt cell lines, the effect of the dual PI3K/mTOR inhibitor PKI-587 was superior to inhibition by Everolimus (RAD001), which solely targets mTOR.

Nonetheless, our preliminary results point to different mechanisms of PKI-587 in the two cell lines: While apoptosis is stronger induced in the well differentiated NCI-H727 cell lines, Western Blot analysis showed that in the SCLC cell line NCI-H69 4E-BP1/4E-BP1 and direct mTORC1-targets become more inhibited after treatment with PKI-587, pointing to a relevance for proliferation regulation.

PKI-587 is therefore a promising inhibitory substance which should be further investigated in preclinical and clinical trials.

References
1. Zawadziak et al. EndocrineRelatedCancer, 2010
3. Freitag et al. Neuroendocrinology, 2016, in revision

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